THE RELATIONSHIP OF PHALAENOPSIS SPECIES BASED ON GENOME HOMOLOGY

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INTRODUCTION

This study deals with the cytology of eight interspecific hybrids of Phalaenopsis. The plants selected represented intra- and inter-sectional crosses, as the genus has been divided into sections according to various authors. An attempt is made to elucidate the relationship of the species involved.

The Orchidaceae comprised of some 15,000 to 30,000 species (Schweinfurth, 1959) form a highly variable family, which has been divided into different numbers of taxa of lower rank. Schlechter (1926) recognized 80 subtribes, whereas Dressler and Dodson (1960) in a more natural system proposed 40 subtribes. At the genus and species level the delimitation of the different taxa is not well established. The interfertility of many genera and species has cast much doubt upon the present classification, just as does the occurrence of sterility in some interspecific hybrids.

The classification of the Orchidaceae is based on morphological characters and in particular on floral characteristics. In respect to these features much transition occurs between closely related entities. Hence, the genera and the species are often not well delimited. Phalaenopsis is morphologically well characterized from other genera and the number of species is relatively small. The phylogenetic relationship of the species is not well understood, but most of the species are recognizable on a morphological basis.

During the last two decades additional information has been obtained regarding a natural classification through cytological investigations. Early observations by Woodard (1951) and more recently by Sagawa (1962) and Shindo and Kamemoto (1963b) indicated a somatic chromosome number of 38 and a variable chromosome size for several Phalaenopsis species. Similar observations were made for a total of 17 Phalaenopsis species by Sagawa and Shoji (1968a, 1968b). Study and comparison of karyotypes may contribute knowledge regarding the evolution and relationship of species (Stebbins, 1950). Also, the degree of genome homology determined through meiotic studies of primary hybrids will elucidate degrees of relationships of the parental species.

REVIEW OF LITERATURE

The genus Phalaenopsis was established by Blume in 1825 when he based a new genus on a species previously described as Epidendrum amabile and Cymbidium amabile by Linnaeus and Roxburgh, respectively (Quisumbing, 1941a). It belongs to the Sarcanthinae (Schweinfurth, 1959), a subtribe of the Orchidaceae, which is distinguished by a monopodial growth habit and lateral inflorescences. The number of species of Phalaenopsis varies from 34 (Rolfe, 1886) through 40 (Schlechter, 1927) to 70 Quisumbing, 1958).

Phalaenopsis is well defined from the other Sarcanthine genera by having broad, elliptic, leathery leaves which are attached distichously to a stem having extremely short internodes. The inflorescences are either short or elongated. The flowers are variable in size, the sepals equally narrow elliptic, the lateral petals either similar to the sepals or much broader. The labellum is connected without a hinge to the columnfoot, three lobed and without any spurlike appendage. Variably shaped calli are present between the two lateral lobes, the middle lobe is either hastate with two auricles at the apex or simple, from ovate through broadly ovate to half-circular. The column bears an anther with two notched pollinia.

Phalaenopsis has an Old-World distribution ranging from Assam through Indo-China, Malaya, Philippines and Indonesia to New Guinea (Quisumbing, 1968).

Due to the tremendous diversity among the Phalaenopsis species several sections have been established within the genus.

Rolfe (1886) established four sections (Table I) in which he classified 34 species. Pfitzer (1889) maintained the first two sections and renamed the section Esmeralda into Antenniferae. The Stauroglottis section was confined to species having lips with an undivided elongated middle lobe, hence those characterized by a lip with a broadened middle lobe, were placed into a new section, Zebrinae.

It can be noticed from Pfitzer's key (page 6) as translated and modified by Quisumbing (1947) that the latter author stressed the fact that the middle lobe is either smooth or hairy.

The section Antenniferae has been clarified recently and the species has been recognized as synonymous with Doritis pulcherrima as described by Lindley in 1838 (Rolfe, 1917; Holttum, 1963, 1965a, 1965b).

According to Dillon (1956) the section Polychilos was assigned by Smith in 1933 to include species as P. cornu-cervi, P. mannii and P. parishii.

Recently, three species considered by Smith as valid Phalaenopsis species, but distinct by their terete leaves and a spur-like gland in the labellum have been transferred to a new genus Paraphalaenopsis by Hawkes (1963).

Finally, Holttum (1966) transferred a number of Phalaenopsis species having a distinct spur into the genus Kingiella, earlier established by Rolfe (1917).

TABLE I. THE FOUR SECTIONS OF THE GENUS PHALAENOPSIS ACCORDING TO ROLFE

Euphalaenopsis	Proboscidioides	Esmeralda	Stauroglottis
1. P. amabilis	8. P. lowii	9. P. esmeralda	11. P. amethystina
2. P. aphrodite		10. P. antennifera	12. P. stobartiana
3. P. stuartiana			13. P. hebe
4. P. schilleriana			14. P. rosea
5. P. delicata			15. P. deliciosa
6. P. X intermedia			16. P. parishii
7. P. X veitchiana			17. P. pallens
			18. P. reichenbachiana
			19. P. devriesiana
			20. P. cornu-cervi
			21. P. pantherina
			22. P. mannii
			23. P. boxallii
			24. P. violacea
			25. P. valentinii
			26. P. maculata
			27. P. mariae
			28. P. fuscata
			29. P. fasciata
			30. P. lueddemanniana
			31. P. corningiana
			32. P. sumatrana
			33. P. tetraspis
			34. P. speciosa

Only the names of the species as enumerated by Rolfe are given; the varieties of the different species have been omitted. (From the Gardeners Chronicle, Vol. 26, 1886).

	Section
1. Petals much broader than the sepals and contracted at the base.	
a. Middle lobe of lip with two cirrhi or two divaricate lobes at the apex; without proboscislike rostellum	Euphalaenopsis
b. Middle lobe of lip without apical appendages; with proboscislike rostellum	Proboscidioides
2. Petals equal to, rarely smaller than sepals; middle lobe of lip entire, without apical appendages and without proboscislike rostellum	
a. Claw of lip without appendages	
(1) Middle lobe of lip ovate; upper surface smooth	Stauroglottis
(2) Middle lobe of lip oblong; upper surface with a crest of hairs	Zebrinae
b. Claw of lip with a pair of slender appendages	Antenniferae

Key to sections of Phalaenopsis according to Pfitzer (1889), as translated and slightly modified by Quisumbing (1947).

A somatic chromosome number of 38 was established for Phalaenopsis by Woodard (1951), Sagawa (1962), Shindo and Kamemoto (1963b) and Sagawa and Shoji (1968a, 1968b).

Woodard (1951) noticed a divergence in chromosome size among the various Phalaenopsis species. This feature was elaborated by Shindo and Kamemoto (1963b) by a comparative study of the somatic chromosomes of seven Phalaenopsis species. A similar study was done by Sagawa and Shoji (1968b) for a total of seventeen species. Both groups of investigators established karyotypes for each of the species studied. The S % value $\left(\frac{\text{length of shortest chromosome}}{\text{length of longest chromosome}} \times 100\%\right)$ and the mean F % value $\left(\sum_{x=1}^{38} \left[\frac{\text{length of short arm of chromosome}_x}{\text{total length chromosome}_x} \times 100\% \right] \times \frac{1}{38}\right)$ for eight Phalaenopsis species as established by Sagawa and Shoji (1968b) have been recorded in Table II. None of the karyotypes is characterized by completely similar values, although the species of the Euphalaenopsis section have similar karyotypes.

TABLE II. VALUES CHARACTERIZING KARYOTYPES

Section	Species	Average chr. length in u	Mean F % value	S % value
Euphalaenopsis	P. amabilis	1.76	41	50
	P. aphrodite	1.56	46	50
	P. sanderiana	1.46	47	67
	P. stuartiana	1.46	42	50
Stauroglottis	P. equestris	2.07	42	47
Zebrinae	P. lueddemanniana	2.13	45	38
Polychilos	P. mannii	3.47	45	40
---	P. amboinensis	2.34	42	23

Phalaenopsis species arranged according to the classification of Pfitzer and Smith. Abstracted from Shoji and Sagawa, 1968b.

MATERIALS AND METHODS

The following plant specimens from the collection at the University of Hawaii were studied:

Hybrid			Collection Number
Phalaenopsis amabilis	x	*Phalaenopsis stuartiana*	UH 1214
Phalaenopsis equestris	x	*Phalaenopsis sanderiana*	UH 1689
Phalaenopsis X *intermedia*			UH 1215
Phalaenopsis lueddemanniana	x	*Phalaenopsis mannii*	UH 1690
Phalaenopsis amboinensis	x	*Phalaenopsis mannii*	UH 1786-1
Phalaenopsis lueddemanniana	x	*Phalaenopsis equestris*	UH 1686
Phalaenopsis mannii	x	*Phalaenopsis equestris*	UH 1638
Phalaenopsis amboinensis	x	*Phalaenopsis sanderiana*	UH 1637

A close correlation between bud size and stage of pollen genesis was found. Table III presents the length of the buds in mm in which metaphase I configurations and microspore division were observed.

The methods for preparation of slides were as described by Shindo and Kamemoto (1962, 1963) with slight modifications. For studies of somatic chromosomes active growing roottips (1.0 - 1.5 mm) were pretreated in an aqueous solution of 0.002 M 8 hydroxyquinoline for 5 hours at 15 - 18^{o} C,

TABLE III. BUD LENGTH (mm) AT METAPHASE I (M_I) AND MICROSPORE DIVISION (MD) FOR EIGHT INTERSPECIFIC PHALAENOPSIS HYBRIDS

Hybrid		Length of buds (mm) M_I	MD
P. amabilis	x P. stuartiana	7.5	9.7
P. equestris	x P. sanderiana	6.8	8.7
P. X intermedia		6.2	8.0
P. lueddemanniana	x P. mannii	8.3	--
P. amboinensis	x P. mannii	9.5	--
P. lueddemanniana	x P. equestris	8.9	11.0
P. mannii	x P. equestris	8.6	10.1
P. amboinensis	x P. sanderiana	10.4	13.9

-- = not observed

fixed in a modified Carnoy mixture (1 part chloroform, 1 part 100% ethyl alcohol, 2 parts glacial acetic acid) for 30 minutes at room temperature, macerated at 60° C in 1 N HCl for 2 - 3 minutes and transferred to 45% acetic acid for 10 minutes. After removal of the rootcap and fractionation, the tissue was stained in 1% aceto-orcein and squashed. For studies of meiosis and microspore division the length of the bud was measured with a vernier calliper in millimeters. The anthercap with the pollinia was removed from the column, cut to expose the pollinia, fixed at 15° C in modified Carnoy mixture for 5 minutes and transferred to 45% acetic acid for 10 minutes at 15° C. The pollen material was separated from the surrounding tissue, spread, stained in 1% aceto-orcein and squashed.

Root tips collected at 11:00 a.m. showed distinctive somatic chromosomes, while buds collected at 10:30 and 11:15 a.m. provided the most favorable material for the observation of metaphase I and microspore division, respectively.

Photomicrographs were taken with a Zeiss Photomicroscope with appropriate lens combination and printed, except if stated otherwise, at a magnification of 2000 times.

OBSERVATIONS AND DISCUSSION

Observations and discussion of the results are arranged as follows:

1. That dealing with somatic chromosomes.
2. That dealing with meiosis and microspore division.
3. A general discussion of the implications of the results on the genus *Phalaenopsis*.

1. Somatic chromosomes

For all hybrids except *P. amboinensis* x *P. mannii* from which no active root tips were available, a number of 38 somatic chromosomes was recorded (Table IV).

The somatic chromosomes of the hybrids *P. amabilis* x *P. stuartiana* (Fig. 1), *P. sanderiana* x *P. equestris* (Fig. 2) and *P.* X *intermedia* (Fig. 3) did not show any unusual features. The chromosomes of the parent species have apparently a similar size. This observation is supported by the findings of Sagawa and Shoji (1968b) who noticed that the karyotypes of the species mentioned here have a similar average chromosome size (Table II).

The somatic chromosomes of the hybrid *P. lueddemanniana* x *P. mannii* fall into two classes by size (Fig. 4), which are quite distinct. Based on the observations of previous investigators it is evident that the longer chromosomes comprise the P. mannii genome, and the shorter ones the *P. lueddemanniana* genome.

TABLE IV. SOMATIC CHROMOSOME NUMBER IN SEVEN INTERSPECIFIC PHALAENOPSIS HYBRIDS

Hybrid		Somatic Chromosome Number
P. amabilis	x P. stuartiana	38
P. sanderiana	x P. equestris	38
P. X intermedia		38
P. lueddemanniana	x P. mannii	38
P. lueddemanniana	x P. equestris	38
P. mannii	x P. equestris	38
P. amboinensis	x P. sanderiana	38

The somatic chromosomes of the hybrids P. lueddemanniana x P. equestris (Fig. 5), P. mannii x P. equestris (Fig. 6) and P. amboinensis x P. sanderiana (Fig. 7) fall into different classes by size also. By comparison with the somatic chromosomes shown in Figures 1, 2 and 3 it can be deduced that the longer chromosomes shown in Figures 5, 6 and 7 are inherited from P. lueddemanniana, P. mannii and P. amboinensis, respectively. The relative size differences among the somatic chromosomes correspond well with the observations and measurements of Sagawa and Shoji (Table I).

2. Meiosis, tetrad formation and microspore division

Based on observations of cytological features and analysis of the metaphase I configurations (Fig. 8, 10) observed in the hybrids P. amabilis x P. stuartiana, P. sanderiana x P. equestris and P. X intermedia (a natural hybrid of P. aphrodite and P. equestris) (Sanders, 1961), it is concluded that these hybrids cannot be distinguished on a cytological basis.

The mean number of bivalents observed in the three hybrids are 18.96, 18.97, and 19.00, respectively, all very similar values (Table V). Even a closer observation of the bivalents, resulting in a comparison of ring- and rodbivalents, did not provide any indication in respect to possible differences. The mean number of ringbivalents observed were for the three hybrids 10.96 9.85 and 10.25 respectively (Table V). Very low mean numbers (0.08 and 0.07 respectively, (Table V) of univalents were observed. The formation of univalents resulted from precocious division of one bivalent.

TABLE V. MEAN AND RANGE OF NUMBER OF CHROMOSOME CONFIGURATIONS AT METAPHASE I IN EIGHT INTERSPECIFIC PHALAENOPSIS HYBRIDS

Hybrid	Bivalents					Univalents		No. of PMC
	Ringbivalent		Rodbivalent		Mean Total			
	Mean	Range	Mean	Range		Mean	Range	
P. amabilis P. x stuartiana	10.96	7-13	8.00	6-12	18.96	0.08	0-2	27
P. sanderiana P. x equestris	9.85	7-12	9.12	7-13	18.97	0.07	0-2	29
P. X intermedia	10.25	9-11	8.75	8-10	19.00	--	--	18
P. lueddemanniana P. x mannii	17.30	16-19	1.70	0-3	19.00	--	--	20
P. amboinensis P. x mannii	11.64	10-15	7.28	4-9	18.92	0.16	0-2	25
	Bivalents							
	Mean	Range						
P. lueddemanniana P. x equestris	15.00	2-8			5.00	28.00	22-34	18
P. mannii P. x equestris	4.88	1-9			4.88	28.24	20-36	8
P. amboinensis P. x sanderiana	12.13	4-19			12.13	13.73	0-30	15

Meiosis resulted in a formation of 100% tetrads (Table VI) in P. amabilis x P. stuartiana, P. sanderiana x P. equestris and P. aphrodite x P. equestris. Microspore division (Fig. 9) did not show any unusual features.

From the analysis and observations it is concluded that there is a complete genome homology within this group of hybrids and that the parental species are cytotaxonomically closely related.

It appears from Table V that there is complete genome homology in the hybrid P. lueddemanniana x P. mannii as a mean number of 19.00 bivalents were observed. The mean number of ringbivalents was very high (17.30) and the number varied only from 16 to 19.

Cytological evidence that bivalent formation between chromosomes of unequal size occurs is provided by some peculiar bivalents. A rod-bivalent indicated by a long arrow in Figure 11 is formed by a long and a short chromosome. Another (short) arrow indicates an "asymmetrical" ringbivalent consisting of two chromosomes of dissimilar lengths. Figure 12 shows next to some of the "asymmetrical" ringbivalents several "frying-pan" shaped ringbivalents. The degree of genome homology indicates a close relationship of P. lueddemanniana and P. mannii.

Another deduction which can be made follows from the observation of the high mean (17.30 number of ringbivalents. As ringbivalents are most likely to be formed by two chromosomes with median or submedian centromeres

one may conclude that about 17 chromosomes of each genome of the respective species are at least acrocentric or metacentric. A preponderance of more or less subtelocentric chromosomes would result in the formation of more robbivalents.

Unfortunately, neither sporad formation nor microspore division could be observed due to the sparse flowering habit of the specimen plant.

The hybrid P. amboinensis x P. mannii exhibited a high degree of genome honology as indicated by the average number of 18.92 bivalents (Table V). In this case a number of 7.28 rodbivalents was observed. Figure 13 presents a number of 19 bivalents at metaphase I. It is concluded that P. amboinensis and P. mannii are closely related.

The previous conclusion drawn from the observations on P. lueddemanniana x P. mannii that probably 17 chromosomes in the P. mannii genome are more or less metacentric to acrocentric, leads, in combination with the observation of an average number of 7.28 rodbivalents in the hybrid P. amboinensis x P. mannii, to the conclusion that about 7 minus 2 chromosomes in the P. mannii genome might be approximately subtelocentric.

Table VI shows that meiosis leads to the regular formation of tetrads only. Chromosomes in microspore division (Fig. 14) exhibited their variable lengths quite well.

The last three hybrids, P. lueddemanniana x P. equestris, P. mannii x P. equestris and P. amboinensis x P. sanderiana are the results of

TABLE VI. DISTRIBUTION OF TYPES OF SPORADS IN PER CENT FOR EIGHT INTERSPECIFIC PHALAENOPSIS HYBRIDS

Hybrid	Te	Te + 1mc	Te + 2mc	Tr + 1mc	Tr + 2mc	Dy	Dy + 1mc	Dy + 2mc	No. Sporad
P. amabilis x P. stuartiana	100	-	-	-	-	-	-	-	100
P. sanderiana x P. equestris	100	-	-	-	-	-	-	-	75
P. X intermedia	100	-	-	-	-	-	-	-	75
P. lueddemanniana x P. mannii	no material available								-
P. amboinensis x P. mannii	100	-	-	-	-	-	-	-	50
P. lueddemanniana x P. equestris	27.5	24.6	5.8	2.9	2.9	20.3	7.3	8.7	69
P. mannii x P. equestris	35.2	45.7	15.6	-	3.5	-	-	-	57
P. amboinensis x P. sanderiana	51.4	32.8	12.5	-	3.3	-	-	-	64

Te = tetrad, Tr = triad, Dy = dyad, mc = microcyte.

hybridization of species with long chromosomes and species with short chromosomes. The differences in chromosome sizes are evident in Figures 5, 6 and 7. In respect to analysis of the meiotic studies no distinction has been made between ring- and rodbivalents. The bivalents observed are intermediate in size when compared with the bivalents in for example P. sanderiana x P. equestris and on the other hand in P. amboinensis x P. mannii. Cytological observations were hampered by the occurrence of chromosomal "stickiness". The chromosomes were less distinct in comparison with the chromosomes observed in the hybrids discussed before.

A mean number of 5.00 bivalents was recorded in P. lueddemanniana x P. equestris, while the number of bivalents varied from 2 to 8. A high mean number of univalents (28.00) occurred (Table V). Figure 15 shows a pollen mother cell in metaphase I showing 26 univalents and 6 bivalents. Some of the bivalents have been indicated by arrows. It is concluded that P. lueddemanniana and P. equestris exhibit poor genome homology.

The types of sporads formed were variable. A total of about 58% tetrads (with or without microcytes) was recorded. (Table VI). Four spores with nuclei of about equal size were recorded as tetrads. Microcytes are spores with a very low number of chromosomes and consequently with small nuclei. Striking is the high number of dyads (with or without microcytes) of about 36% (Table VI). Kamemoto and Shindo (1962) also noticed the high percentage of dyad formation in cases with poor genome homology. Dyad formation resulted

from the formation of restitution nuclei after telophase I followed by a normal equational division. Figure 16 presents a dyad and a tetrad with two microcytes.

The mean number of bivalents observed in P. mannii x P. equestris was 4.88, a value almost similar to the value recorded for the hybrid P. lueddemanniana x P. mannii (Table V). It is concluded that the genome of P. equestris displays the same degree of homology towards the genome of P. mannii as to the genome of P. lueddemanniana.

Cytological observations indicated that the chromosomes were frequently arranged in the vicinity of the equatorial plane (Fig. 17). This phenomenon may account for the high percentage (96.5) of tetrads, with or without microcytes, observed (Table VI).

P. amboinensis x P. sanderiana exhibited a mean number of 12.13 bivalents. Moreover, the number of bivalents showed a very large range from 4 to 19 (Table V). Figures 18 and 19 show 9 bivalents + 20 univalents and 19 bivalents respectively, at metaphase I. It might be possible that some of the configurations are "pseudobivalents". Pseudobivalents are formed by two chromosomes lacking strong homology held together by a matrix connection instead of chiasmata (Walters, 1954). Person (1955) has provided cytological evidence with haploid wheat that side-by-side associations lacking chiasmata are formed by homologous chromosomes, whereas end-to-end associations without chiasmata are formed by non-homologous chromosomes. Kamemoto and Shindo (1962) considered the occurrence of pseudobivalents as an indication of

a certain degree of homology. Therefore, it is concluded that the genomes of P. amboinensis and P. sanderiana exhibit a fair degree of homology.

The fairly high degree of homology resulted in the formation of a high percentage (96.7) of tetrads with or without microcytes (Table VI). The formation of a relatively high number of bivalents is followed by a more or less regular telophase I (Fig. 20) resulting, finally, in the formation of a tetrad with some microcyte (Fig. 21).

3. General discussion

On the basis of the cytological observations as discussed in the previous sections it appears justifiable to separate the eight parent species of the eight Phalaenopsis hybrids studied into two groups. The first group would include P. amabilis, P. stuartiana, P. sanderiana, P. aphrodite and P. equestris. The second group would include P. mannii, P. lueddemanniana and P. amboinensis. Although all possible hybrids which might result from hybridization of the various species in all possible combinations have not been studied, it is postulated that there is a complete genome homology within each group. It seems to be reasonable to assume, for example, that P. amboinensis and P. lueddemanniana would exhibit genome homology, considering the genome homology as found in the hybrid of P. lueddemanniana and P. mannii and, on the other hand, in the hybrid of P. amboinensis and P. mannii. A similar reasoning would apply to the species within the first group.

As far as the cytotaxonomical relations between the two groups of Phalaenopsis species is concerned, there are two indications:

The first is the poor genome homology recorded for P. equestris with the two species of the second group, P. lueddemanniana and P. mannii; the second is the fairly high genome homology recorded for P. sanderiana and P. amboinensis. This indicates that P. amboinensis of the second group of species is apparently most closely related to the species of the first group. The results as discussed suggest that the classification of the various species the different sections as presented in the following table do not appear to be natural.

Species	Section	Author
P. amabilis P. aphrodite P. sanderiana P. stuartiana	Euphalaenopsis	Rolfe, Pfitzer
P. equestris	Stauroglottis	Rolfe, Pfitzer
P. lueddemanniana	Stauroglottis Zebrinae	Rolfe Pfitzer
P. mannii	Stauroglottis Polychilos	Rolfe Smith
P. amboinensis	not classified	--

P. equestris would have to be taken out of the Stauroglottis section and grouped together with the "Euphalaenopsis" species. P. lueddemanniana,

P. mannii and P. amboinensis could be united into one section. In fact, this appears to be a revaluation of the system proposed by Rolfe. His section Esmeralda is not valid (Rolfe, 1917; Holttum, 1963) and the situation, pertaining to the section Proboscidiodes with a single species P. lowii, needs to be clarified (Quisumbing, 1968). The system proposed by Pfitzer and Smith seems to have validity for analytical purposes only and it is not natural.

A striking parallel is found between the division of the genus into two apparently natural groups and the distribution of the species. According to Quisumbing (1958) all species of the first group are found in the Philippines, whereas P. amabilis has even a larger distribution as it is also found in Java, Borneo and the Sunda islands. The species of the second group, however, have, excepting P. lueddemanniana, a disjunct distribution. P. mannii is found in Assam at the periphery of the area in which Phalaenopsis species occur. P. amboinensis is confined to Amboina and P. lueddemanniana, a variable species in respect of flower-color, is found throughout the Philippines.

The karyotype studies by the different authors (Shindo and Kamemoto, 1963b; Sagawa and Shoji, 1968b) indicated only that the karyotype of P. equestris is very similar to the karyotypes of the species of the Euphalaenopsis section. All other species investigated have distinct karyotypes. Shindo and Kamemoto recognized from their karyotype studies two groups; one group confined to the Philippines comprising some studied "Euphalaenopsis" species and P. lueddemannia, besides another group of extra-Philippine species as

P. mannii and P. violacea. The present study confirms this division, however, a further separation of P. lueddemanniana from the species of the Euphalaenopsis section seems to be justified.

Study of genome homology appears to be extremely useful for establishing the relationship of the species of Phalaenopsis. The classification of the species within the groups according to a possible evolutionary scheme has to be based on karyotype studies, however. The relation between karyotype development and evolution has been established by Stebbins (1950). Karyotypes having a strong divergence in size ("asymmetrical" karyotypes) are most advanced. Expressed in S % value an evolved karyotype would exhibit a low value. Changes in chromosome size may proceed in any direction; from small to large or from large to small.

Shindo and Kamemoto (1963a, 1962b) postulated that a karyotype condition as established for P. lueddemanniana might represent the primitive condition in Phalaenopsis as the size of the chromosomes of P. lueddemanniana is similar to the chromosome sizes as found in other related genera.

Using the S % values (Table II) as criteria of "primitiveness" the species in the two groups can be arranged as shown in the figure on page 26, in which P. lueddemanniana has been placed in the centre.

The conclusions regarding the number of chromosomes with median and sub-median centromeres in the genomes of P. lueddemanniana, P. mannii and P. amboinensis are consistent with the observations on the

karyotypes of these species by Sagawa and Shoji (1968b). According to these authors the first two species do not possess any chromosome with subterminal centromeres. P. amboinensis has a bimodel karyotype with 6 or 7 pairs of smaller chromosomes having a size similar to the size established for the smallest chromosomes of the species of the first (Euphalaenopsis) group. The occurrence of the mean number of 7.28 rodbivalents recorded in the hybrid P. amboinensis x P. mannii might well coincide with the number of small chromosomes established in the genome of P. amboinensis, but these small chromosomes are not subtelocentric as was postulated earlier.

The scheme as presented in the figure on page 26 may be supported as further investigations of other hybrids become available. The possible evolutionary link between the two natural groups might therefore be established.

Phalaenopsis mannii (3.47u, S % = 40)

Phalaenopsis lueddemanniana (2.13u, S % = 38)

Phalaenopsis amboinensis (2.34u, S% = 23)

Phalaenopsis sanderiana (1.46u, S % = 67)

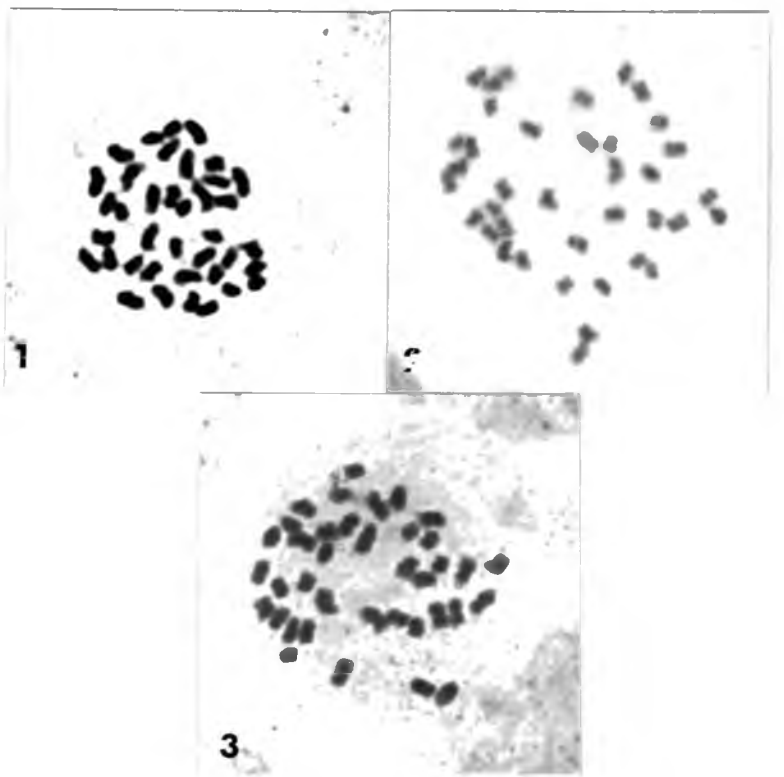
Phalaenopsis amabilis (1.76u, S % = 50)

Phalaenopsis aphrodite (1.56u, S % = 50)

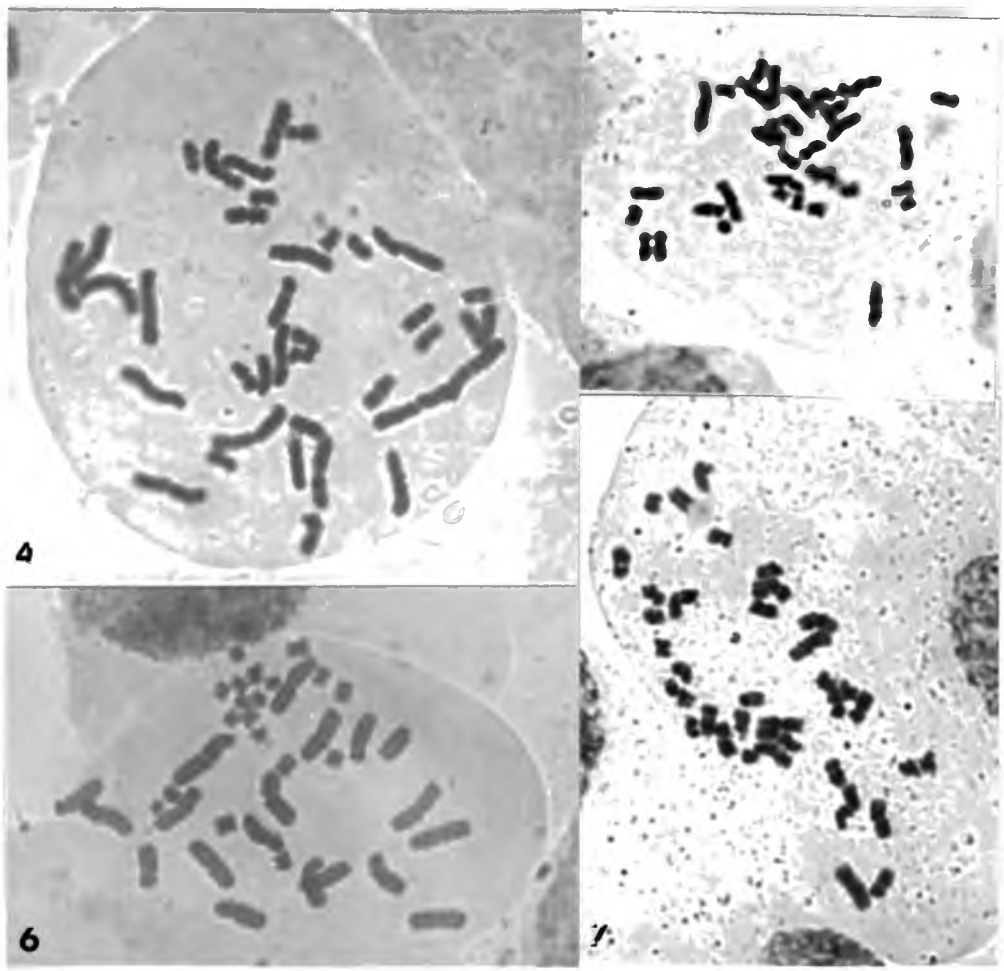
Phalaenopsis stuartiana (1.46u, S % = 50)

Phalaenopsis equestris (2.07u, S % = 47)

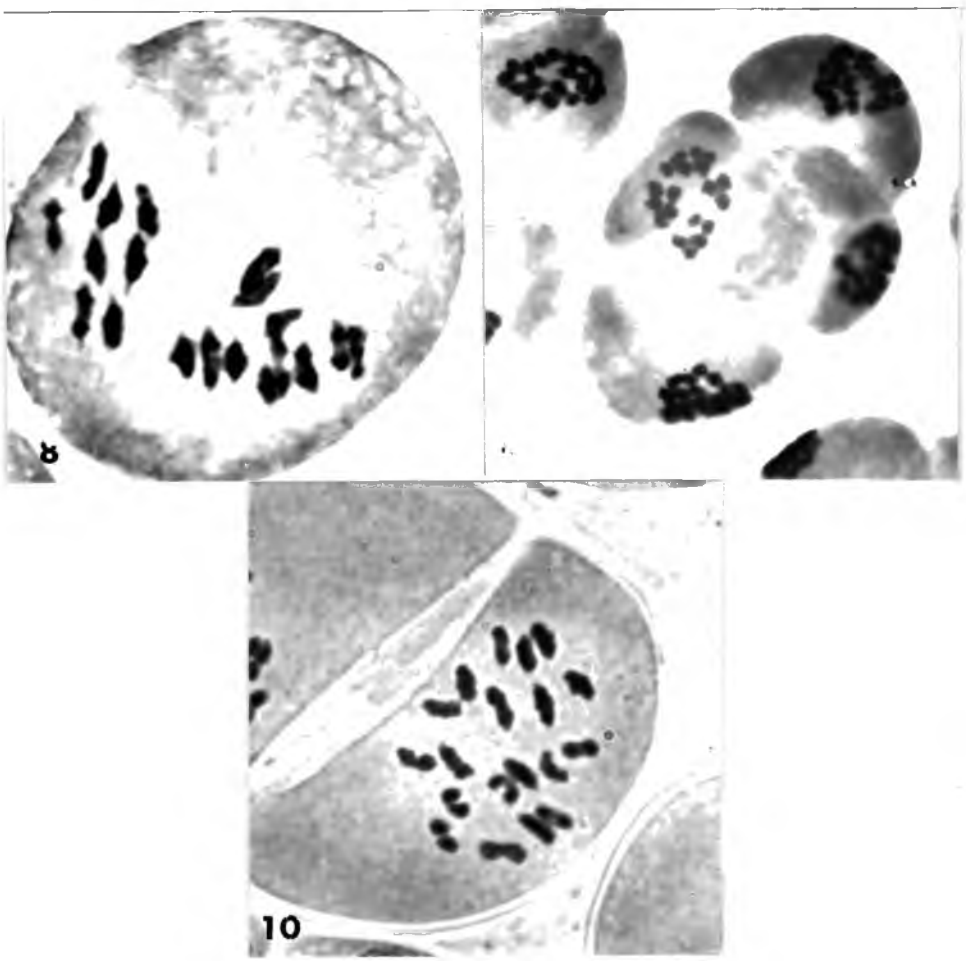
The division of eight species of *Phalaenopsis* into two groups based on genome homology, average chromosome lengths and S % values for the different karyotypes.



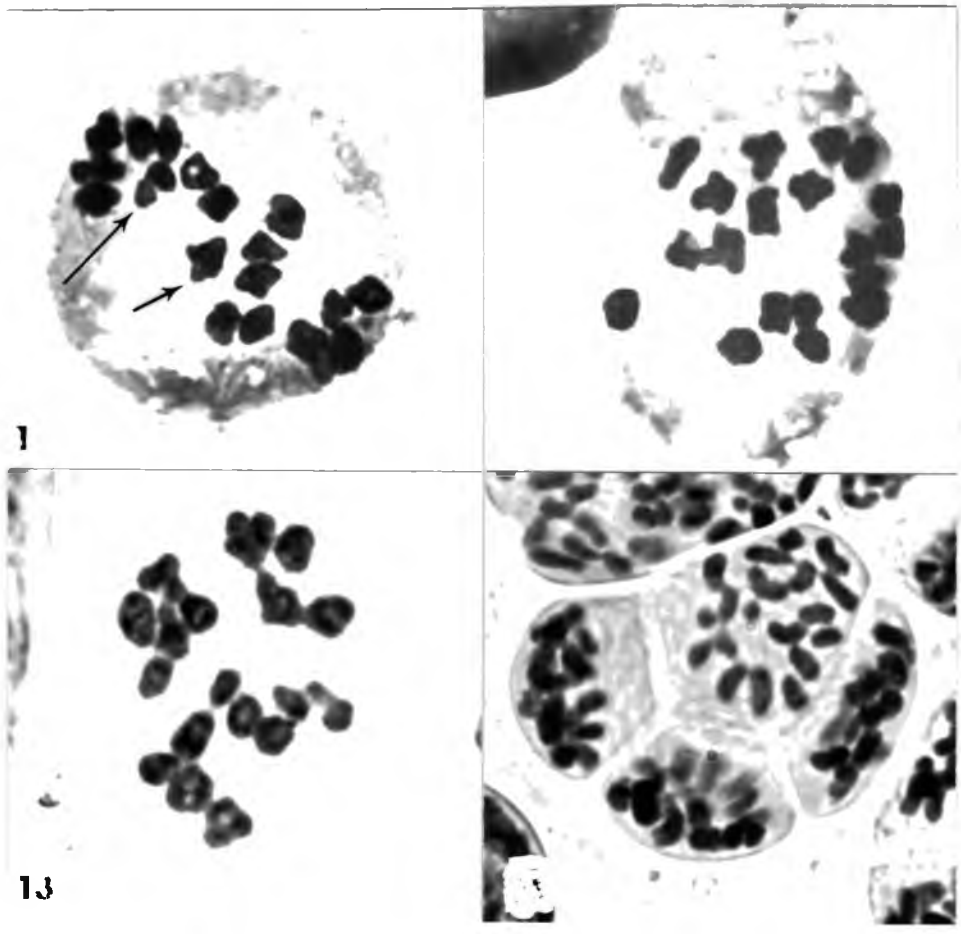
Figures 1 - 3; Somatic chromosomes of interspecific hybrids of Phalaenopsis, x 2000, --- Figure 1; P. amabilis x P. stuartiana, --- Figure 2; P. sanderiana x P. equestris, --- Figure 3; P. X intermedia.



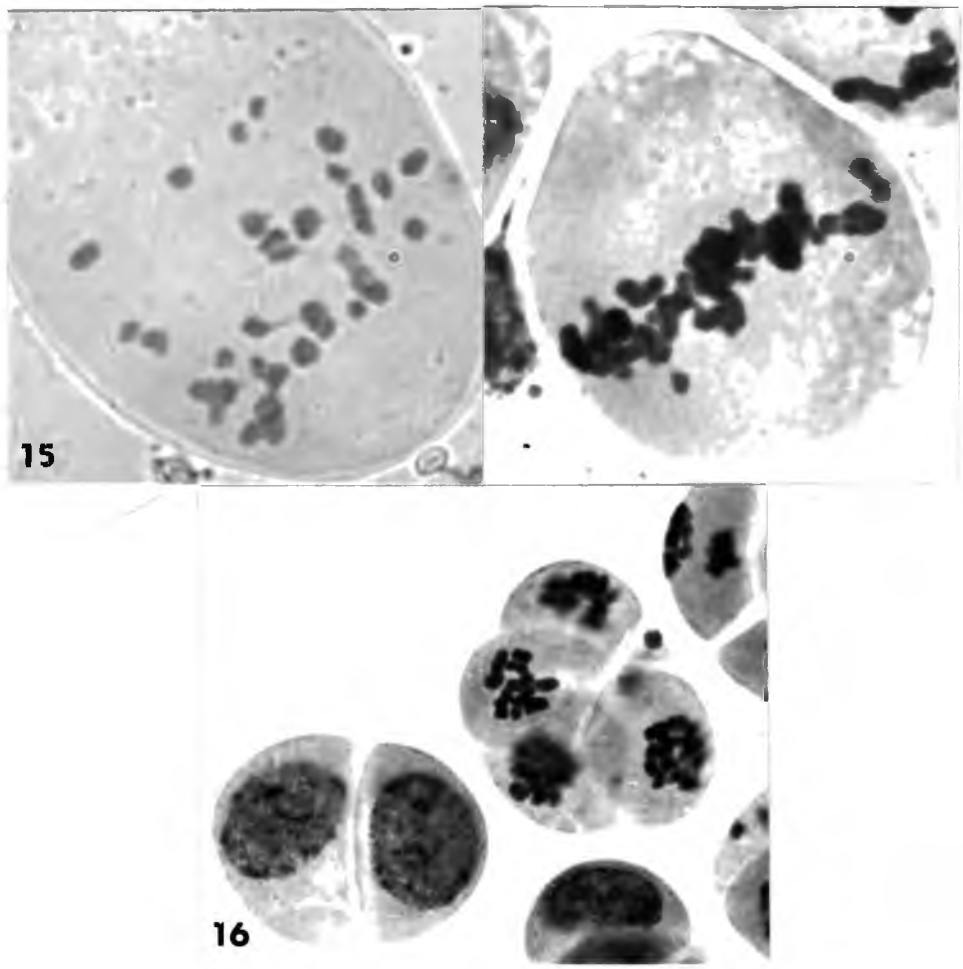
Figures 4 - 7; Somatic chromosomes of interspecific hybrids of Phalaenopsis, x 2000, --- Figure 4; P. lueddemanniana x P. mannii, --- Figure 5; P. lueddemanniana x P. equestris, --- Figure 6; P. mannii x P. equestris, --- Figure 7; P. amboinensis x P. sanderiana, --- In figures 4, 5, 6 and 7 the longer chromosomes are from the genomes of respectively P. mannii, P. lueddemanniana, P. mannii and P. amboinensis.



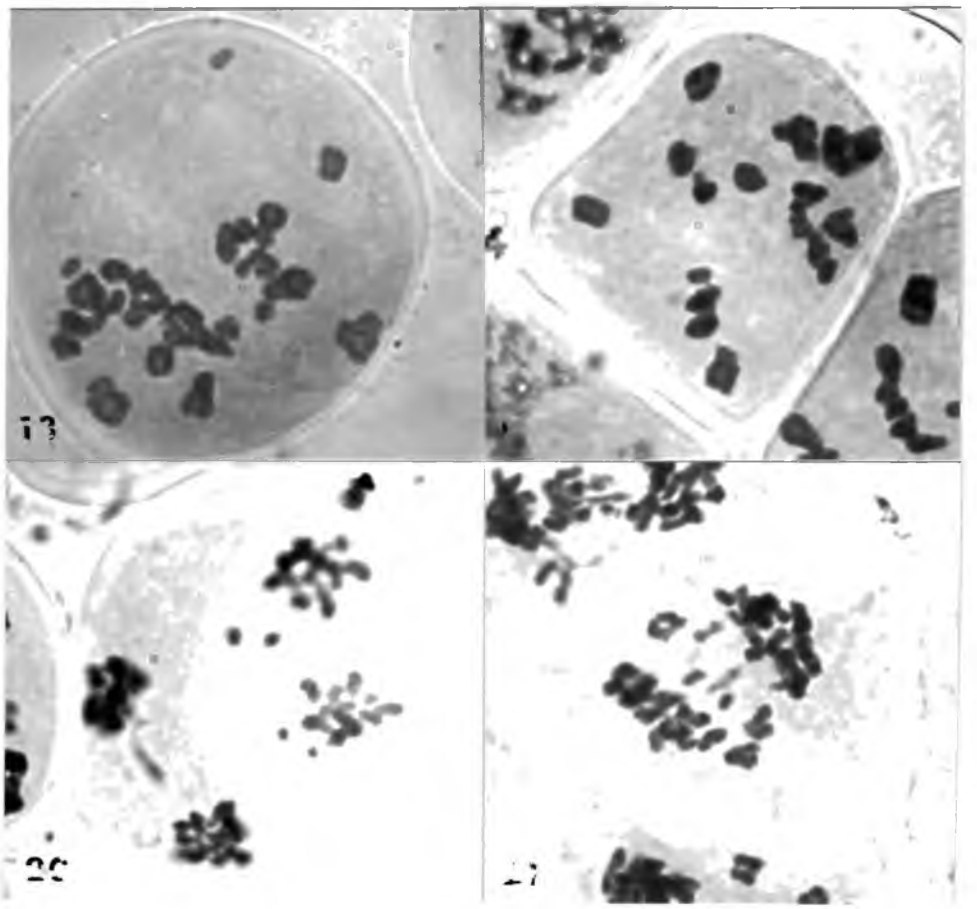
Figures 8 - 10; Chromosomes of interspecific hybrids of Phalaenopsis, x 2000 --- Figure 8; P. amabilis x P. stuartiana; 19 bivalents at Metaphase I, --- Figure 9; P. amabilis x P. stuartiana, tetrad with 19 chromosomes in each microspore, --- Figure 10; P. sanderiana x P. equestris, 19 bivalents at metaphase I.



Figures 11 - 14; Chromosomes of interspecific hybrids of Phalaenopsis, x 2000, --- Figure 11, 12; P. lueddemanniana x P. mannii, --- Figures 13, 14; P. amboinensis x P. mannii, --- Figure 11; 19 bivalents at metaphase I, the long arrow indicates a rodbivalent formed by a short and a longer chromosome, the short arrow indicates a ringbivalent formed by two chromosomes of unequal lengths, --- Figure 13; 19 bivalents at metaphase I, --- Figure 14; tetrad with 19 chromosomes visible in one of the microspores.



Figures 15 - 17; Chromosomes of interspecific hybrids of Phalaenopsis, --- Figures 15, 16; P. lueddemanniana x P. equestris, --- Figure 15; 6 bivalents + 26 univalents, --- Figure 16; one dyad and one tetrad with microcytes, --- Figure 17; P. mannii x P. equestris, chromosomes more or less aligned at the equatorial plane at metaphase I. Figures 15, 17 x 2000, Figure 16, x 1500.



Figures 18 - 21; Chromosomes of interspecific hybrids of Phalaenopsis, x 2000, --- Figures 18, 19, 20 and 21; P. amboinensis x P. sanderiana, --- Figure 18; 9 bivalents + 20 univalents at metaphase I, --- Figure 19, 19 bivalents at metaphase I, --- Figure 20; telophase I, some chromosomes lagging at the equatorial plane, --- Figure 21; telophase II.

SUMMARY

The purpose of this investigation was to establish the relationship of eight species of Phalaenopsis based on genome homology.

On the basis of floristic characters Phalaenopsis has been divided previously by various taxonomists into a variable number of sections. More recently it has been established that several species are distinct by their karyotypes also.

The species with their sections included in this investigation were: P. amabilis, P. aphrodite, P. sanderiana, P. stuartiana (Euphalaenopsis section); P. equestris (Stauroglottis section); P. lueddemanniana (either Stauroglottis or Zebrinae section); P. mannii (either Stauroglottis or Polychilos section); and P. amboinensis (section not cited in literature).

In the hybrids of the first five species a complete genome homology was observed. It was not possible to distinguish the hybrids on a cytological basis. In the hybrids of the latter three species complete genome homology was also observed. Observations indicated that rod- and ringbivalents consisting of chromosomes of dissimilar lengths occur. From the configurations observed in the hybrids the morphology of the somatic chromosomes of the parent species could be predicted. The hybrids P. lueddemanniana x P. equestris, P. mannii x P. equestris and P. amboinensis x P. sanderiana showed respectively minor genome homology in the first two hybrids and a fair homology in the third hybrid.

It was concluded that the species involved can be separated into two apparently natural groups in which complete genome homology occurs. The sections recognized earlier do not appear to be natural.

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